EPA/OPP MICROBIOLOGY LABORATORY
ESC, Ft. Meade, MD

Standard Operating Procedure for AOAC Sporicidal Activity Test (*Bacillus* species)

SOP Number: MB-15-00

Date Revised: 06-25-03

Prepared By: _____ Date: ___/___/___

Print Name:_____

Reviewed By: _____ Date: ___/___/___

Print Name:_____
Technical Staff

_____ Date: ___/___/___

Print Name:_____
QA Officer

_____ Date: ___/___/___

Print Name:_____
Laboratory Director

Date Issued: ___/___/___

Withdrawn By: _____ Date: ___/___/___

Controlled Copy No.: _____

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1.0 SCOPE AND APPLICATION:

1.1 This SOP is based on the AOAC Official Method 966.04, Sporicidal Activity of Disinfectants (AOAC 17th Edition), and is suitable for determining the presence or absence of sporicidal activity of liquid sporicidal agents against aerobic spore-forming bacteria of the genus *Bacillus*. In most cases, *Bacillus subtilis* (ATCC #19659) will be the test microbe selected for sporicidal testing; however, if requested, other *Bacillus* species may also be used.

2.0 DEFINITIONS:

2.1 AOAC = AOAC INTERNATIONAL

2.2 CFU = Colony Forming Unit

2.3 DI = Deionized Water

2.4 FTM = Fluid Thioglycollate Medium

2.5 PBDW = Phosphate Buffered Dilution Water

2.6 TNTC = Too Numerous to Count

2.7 TSA = Tryptic Soy Agar

3.0 HEALTH AND SAFETY:

3.1 All manipulations of the test organism (*Bacillus subtilis*) are required to be performed in accordance with biosafety practices stipulated in SOP MB-01, Laboratory Biosafety. Biosafety level 2 practices will be followed for tests involving *Bacillus subtilis*; however, the appropriate biosafety practices must be addressed for individual microbes.

3.2 Sporicidal agents may contain a number of different active ingredients such as peracetic acid, chlorine, and peroxides. Latex gloves and other personal protective clothing or devices must be worn during the handling of these items for purposes of activation, dilution, or efficacy testing. A chemical fume hood or other containment equipment is employed when performing tasks with concentrated products.

4.0 CAUTIONS:

4.1 To ensure the stability of a diluted sporicidal agent, prepare the dilutions within four hours of the disinfectant treatment step unless label claims indicate otherwise.

4.2 Strict adherence to the protocol is necessary for the validity of test results.

4.3 Use appropriate aseptic techniques for all test procedures involving the manipulation of the test organisms and associated test components.

5.0 INTERFERENCES:

5.1 Touching the interior sides of the medication tube should be avoided while the carriers are being lowered into the sporicidal agent and the hook is being removed. Contact with the interior sides of the medication tube may cause adhesion of spores which are not in contact with the sporicidal agent. This may result in re-inoculation of the carriers with spores as they are being removed from the medication tube. Re-inoculation of the carriers with spores can lead to false positive results.

6.0 PERSONNEL QUALIFICATIONS:

6.1 Personnel are required to be knowledgeable of the procedures in this SOP. Documentation of training and familiarization with this SOP can be found in the training file for each employee.

7.0 SPECIAL APPARATUS AND MATERIALS:

7.1 For *B. subtilis* (ATCC #19659), preinoculated porcelain penicylinders and silk suture loops can be purchased from a commercial vendor that uses documented AOAC methods for spore production, inoculation, and HCl resistance testing. One source is Presque Isle Cultures, 3804 West Lake Rd., P.O. Box 8191, Erie PA 16505.

7.2 Penicylinders - porcelain, 8±1 mm OD, 6±1 mm ID, 10±1 mm length (Fisher Catalog No. 07-907).

7.3 Tissue grinder, Thomas Scientific, No. 3431-E20, size B, or equivalent.

7.4 Suture loops- size 3 silk suture (3, 6.0 metric, silk black braided SA-9G) Uninoculated suture loops that have been extracted with chloroform according to the AOAC method 966-04 B(f) can be purchased from Presque Isle Cultures, 3804 West Lake Rd., P.O. Box 8191, Erie PA 16505.

8.0 INSTRUMENT OR METHOD CALIBRATION:

8.1 Refer to the laboratory equipment calibration and maintenance SOPs (SOP EQ series) for details on method and frequency of calibration.

9.0 SAMPLE HANDLING AND STORAGE:

9.1 Sporicidal agents are stored according to manufacturers' recommendations or at room temperature if the product label does not specify a storage temperature. Those sporicidal agents requiring activation or dilution prior to use will only be activated or diluted within four hours of testing unless label directions specify otherwise.

10.0 PROCEDURE AND ANALYSIS: Listed below are the sub-sections described in this section. Inoculated carriers may be prepared at the testing facility or purchased from a commercial vendor (see Sect. 7.0). Sub-sections 1-5 describe the methods for inoculating carriers and performing the HCl resistance test. The procedure for tying suture loops and performing the chloroform extraction are not described here (see reference 15.1 AOAC Sporicidal Activity Test). Suture loops should be purchased pre-extracted (see Sect. 7.0). Table 1 summarizes the sub-sections to follow based on the particular type of carrier being used in testing.

10.1 Storage and Preparation of Preinoculated Carriers
10.2 Preparation of Suture Loops for Inoculation
10.3 Preparation of Penicylinders for Inoculation
10.4 Preparation of Test Culture
10.5 Inoculation of Spore Carriers
10.6 Determination of Acid Resistance
10.7 Determination of Spore Load
10.8 Sporicidal Agent Sample Preparation
10.9 Testing Procedure
10.10 Testing Controls
10.11 Confirmation Steps

Table 1

Sub-sections to follow based on the type of carrier being used											
If you are starting with...	Sections to Follow										
	10.1	10.2	10.3	10.4	10.5	10.6	10.7	10.8	10.9	10.10	10.11
Pre-inoculated suture loops	×						×	×	×	×	×
Pre-inoculated porcelain carriers	×						×	×	×	×	×
Pre-extracted Unseeded suture loops		×		×	×	×	×	×	×	×	×
Unseeded Porcelain carriers			×	×	×	×	×	×	×	×	×

10.1 Storage and Preparation of Pre-Inoculated Carriers:

10.1.1 Upon receipt of pre-inoculated carriers, assign a tracking number (see SOP QC-09) and store under vacuum. Carriers should be maintained under vacuum and can be used for up to 90 days from date of receipt. After 3 months, a subset of 3 to 6 carriers from the initial set will be returned to Presque Isle Cultures for HCL resistant testing. In addition, the OPP Microbiology Laboratory will conduct a titer enumeration on 3 of the carriers.

10.1.2 On the day of testing, remove petri plates of spore carriers from the vacuum desiccator and transfer carriers with a sterile hook to a double matted (2 layers of filter paper) petri dishes, no more than 30 carriers per petri dish.

10.2 Preparation of Suture Loops for Inoculation:

10.2.1 Place pre-extracted suture loops into petri dishes matted with 2 layers of filter paper in groups of 15 suture loops per petri dish.

10.2.2 Sterilize at 121°C for 20 minutes.

10.2.3 Store sterile carriers at room temperature for up to 3

months. After 3 months, suture loops will be re-autoclaved.

10.2.4 All suture loops used in efficacy testing are discarded after use.

10.3 Preparation of Penicylinders for Inoculation:

10.3.1 Physical Screening: Examine porcelain carriers individually and discard those with scratches, nicks, spurs, or discolorations. Record screening results in the Physical Screening of Carriers Record Form.

10.3.2 Rinse carriers gently in deionized water three times to remove loose material and allow to drain on absorbant material.

10.3.3 Place rinsed porcelain carriers into petri dishes matted with 2 layers of filter paper in groups of 15 carriers per petri dish.

10.3.4 Sterilize at 121°C for 20 minutes.

10.3.5 Store sterile carriers at room temperature for up to 3 months. After 3 months, porcelain carriers will be re-cleaned and autoclaved.

Note: Care should be taken in placing and handling porcelain carriers in Petri dishes to minimize carrier movement and avoid excessive contact between carriers that might result in chips and cracks.

10.3.6 All porcelain carriers used in efficacy testing are discarded after use.

10.4 Preparation of Test Culture:

10.4.1 Initiate test culture by inoculating 10 to 20 (depending on the number of 60 carrier tests to be conducted in one day), 10 mL tubes (25 × 150 mm) of Soil Extract Nutrient Broth (see reference 15.1 AOAC Sporicidal Activity Test) with one loopful of inoculum from a monthly transfer stock tube of

Bacillus subtilis.

10.4.2 Incubate tubes for 72±2 hours at 37±1°C.

10.5 Inoculation of Spore Carriers:

10.5.1 Pour contents of one tube of the 72 hour culture into a sterile tissue grinder and macerate the pellicle. Filter through a sterile funnel containing moist glass wool or cotton into a sterile container. Repeat for the remaining tubes.

10.5.2 Pool spore filtrates into a separate sterile holding container.

10.5.3 Pipet 10 mL volumes into sterile 25 × 150 mm test tubes agitating holding container between each 10 mL transfer to maintain spores in suspension.

10.5.4 Add 10 sterile penicylinders or 10 sterile suture loops to each tube containing 10 mL of spore filtrate and let stand 10-15 minutes. Carriers should remain completely immersed in the inoculum during the exposure period.

10.5.5 Remove carriers with a sterile hook and place in sterile, double matter (2 layers of filter paper) petri dishes, no more than 30 carriers per petri dish.

10.5.6 Place Petri dishes containing contaminated carriers in a vacuum desiccator containing $CaCl_2$ and draw a vacuum of 69 cm (27") Hg for 20 minutes. Dry spores under vacuum for 24 hours before use. Spore carriers should be maintained under vacuum and can be used for up to 3 months. Carriers may be used after 3 months if they pass the hydrochloric acid resistance test described in section 10.6.

10.6 Determination of Acid Resistance:

10.6.1 Hydrochloric acid (HCl) resistance testing will be performed every 3 months by Presque Ilse Cultures, or as needed at

the OPP Microbiology Laboratory on individual carrier lots.

10.6.2 Equilibrate water bath to 20±1°C.

10.6.3 Pipet 10 mL of 2.5N HCl into two 25 × 100 mm tubes and place them into the water bath. Allow at least 30 minutes to reach temperature equilibrium.

10.6.4 Start timer and rapidly transfer 4 inoculated suture loops or porcelain penicylinders into an acid tube (2.5 N HCl). Do not allow carriers or needle hook to contact the inside wall of acid tube.

10.6.5 Transfer individual suture loops or porcelain peniclyinders after 2, 5, 10, and 20 minutes of HCl exposure to a separate Modified Fluid Thioglycollate tube. Rotate each tube vigorously by hand for 20 seconds and then transfer carrier to a second tube of Modified Fluid Thioglycollate Medium.

10.6.6 Viability Controls: Place one of each type of carrier in a separate tube of Modified Fluid Thioglycollate Medium.

10.6.7 Media Controls: One tube of uninoculated Modified Fluid Thioglycollate Medium is used as a negative medium control.

10.6.8 Incubate all test and control tubes for 21 days at 37±1°C. Record results as growth (+) or no growth (-) at each time period.

10.6.9 Spores should resist HCl for $\geq$2 minutes to be qualified as resistant test spores. Growth must occur in tube containing carrier. Discard carriers if not resistant and repeat preparation of carriers as previously described.

10.7 Determination of Spore Load for Porcelain penicylinders and silk suture loops inoculated with spores of *Bacillus subtilis*: Prior to use, determine the spore load on each lot of carriers. A lot is defined as those carriers prepared at the same time from a single preparation of *Bacillus subtilis* inoculum. For lots of carriers 300 or less, determine the spore load on a minimum of 3 randomly selected carriers. For lots of carriers greater than

300, determine the spore load on a minimum of 1% of the total number of carriers (rounding up the nearest whole carrier, for example, a lot of 405 carriers would require enumerating the spore load on 5 carriers). Carrier counts need only be performed once on each lot of carriers and will apply to all tests using those carriers during the 3 month period in which the carriers are valid and will be independent of acid resistance tests.

10.7.1 Place each inoculated carrier into a 20 × 150 mm tube containing 10 mL of sterile DI water.

10.7.2 Place all tubes with carriers into an appropriately sized beaker and fill the beaker with tap water to the level of sterile DI water in the tubes.

10.7.3 Hold the beaker in the sonicator so that the water level in the beaker is even with the water level fill line on the sonicator tank and fill the tank up with tap water to the water level fill line. Be sure that the water level in the tank never falls below one inch from the top of the tank.

10.7.4 Manually hold the beaker in the sonicator tank so that it is not touching the bottom and so that all three water levels (inside the test tubes, inside the beaker and the sonicator tank) are the same.

10.7.5 Sonicate the samples for 5 minutes.

10.7.6 Following sonication, vortex each tube for 2 minutes.

10.7.7 Dilute the spore suspensions by transferring 1 mL aliquots to tubes containing 9 mL sterile DI water. Dilute the spore suspension out to 10^{-7} and plate 1 mL of dilutions 10^{-4} through 10^{-7}. Plate each dilution in duplicate.

10.7.8 To each plate, add molten TSA that has been tempered to 45-50°C. Swirl the plates to distribute spores evenly in the agar and allow to solidify.

10.7.9 Invert the plates and incubate for 24-48 hours at 37 ±1°C.

Colonies may be counted by hand or with aid of a plate counter. Dilutions yielding between 30 and 300 CFU per plate are used for enumeration. Plates that have colony counts over 300 will be reported as TNTC. Record the results on the appropriate form.

10.7.10 Average spore counts per carrier should be 2×10^5 (based on an EPA/AD recommendation for minimum carrier counts).

10.8 Sporicidal Agent Sample Preparation:

10.8.1 Turn on the recirculating chiller and water bath and allow them to come to 20±1°C or the temperature specified by the sporicidal agent manufacturer.

10.8.2 Ready-to-use sporicidal agents are tested as received; no dilution is required.

10.8.3 Prepare sporicidal agent samples aseptically according to the manufacturers' instructions. Use dilution of the sporicidal agent, contact time, temperature, diluent, organic soil, hard water, and neutralizers are identified in the test protocol. Record test parameter information on the Test Information Sheet (see 16.1).

10.8.4 To ensure stability, prepare dilutions of the sporicidal agent within four hours of performing the assay unless test parameters specify otherwise.

10.8.5 Prepare all dilutions with sterile standardized volumetric glassware.

10.8.6 Prior to opening the container of a liquid sporicidal agent, gently shake the container. Place the container inside the BSC, remove the cap and thoroughly clean the area around the cap and spout with 70% ethanol. Allow the surface to dry. Do not touch the inside surface of the cap. If present, carefully remove the seal attached to the lip of the spout with cooled, flame-sterilized instruments (i.e., razor blade, forceps).

10.8.7 Pour an appropriate aliquot of the sample into a sterile beaker. Do not place a pipette or any other instrument inside the product container. Place cap on the product container and secure tightly. From the beaker, aseptically dispense ready-to-use sporicidal agents directly into sterile medication tubes or initiate dilutions for diluted sporicidal agents (see 10.8.9).

10.8.8 For diluted sporicidal agents, use ≥ 1.0 mL of sample to prepare the use-dilution to be tested. Use v/v dilutions for liquid sporicidal agents and w/v dilutions for solids. Round to two decimal places toward a stronger sporicidal agent. Record sporicidal agent preparation on the Media/Reagent Preparation Sheet (see SOP QC-15, Media Prep and Sterilization Run Numbers).

10.8.9 Dispense 10 mL aliquots of the diluted or ready to use sporicidal agent into fifteen 25 mm × 100 mm medication tubes (12 for testing and 3 extra).

10.8.10 Place medication tubes containing the sporicidal agent in the water bath for approximately 10 minutes to allow for equilibration of the sporicidal agent to the specified temperature.

10.9 Testing Procedure: In brief, inoculated carriers are dropped at 2 minute intervals in groups of five carriers at a time into tubes of sporicidal agent held at 20±1°C or a specified temperature. After the prescribed contact period, the carriers are transferred one at a time into separate tubes of neutralizer (refer to SOP MB-12 Neutralization Confirmation Procedure for Products Evaluated with the AOAC Sporicidal Activity Test [Bacillus Species]) are transferred within each 2 minute interval. After all the transfers have been completed, the carriers are transferred again to fresh tubes of subculture media and incubated for 21 days at 37±1°C. If no growth is observed after the 21 day incubation period, the tubes are heat-shocked at 80±2°C for 20 minutes and incubated an additional 72 hours at 37±°C. Results are reported as growth (+) or no growth (0).

A note about timing: A test of 60 carriers would require 12 tubes of sporicidal agent. Given a 10 minute contact time, the maximum number

of carriers that could be tested in one timed operation would be 25. A 60 carrier test would require three timed events, 2 × 25 carriers, and 1 × 10 carriers. A minimum contact time of 24 minutes would be required to allow for all 12 sets of 5 carriers to be dropped in one continuous timed operation. Modify the number of timed operations to accommodate exposure times that are 10 minutes or less.

10.9.1 Carriers are sequentially transferred at 2 minute intervals in groups of 5 from the petri dish to the medication tubes containing the sporicidal agent using a sterile hook.

10.9.2 At two minute intervals add 5 carriers to each tube of sporicidal agent. Immediately after placing the carriers into the medication tube, place it back into the water bath. The carriers must be deposited into the tube within ±5 seconds of the prescribed drop time. Flame the hook and allow it to cool after each carrier transfer.

10.9.3 Reminder: When lowering the carriers into the medication tubes, neither the carriers nor the wire hook may touch the interior sides of the tube. If the interior sides are touched, the tube number is noted on the AOAC Sporicidal Activity Test Results Sheet. If any carrier from that group of 5 yields a positive result, the 5 carrier set will not be counted. Contamination of this type may result in retesting.

10.9.4 At the completion of the exposure period, the carriers are transferred in the same sequential timed fashion into the primary subculture tubes containing the appropriate neutralizer (10 mL in 20 × 150 mm tubes). The carriers are removed one-at-a-time from the sporicidal agent medication tube with a sterile hook, tapped against the interior sides of the tube to remove the excess sporicidal agent, and transferred into the neutralizer tube. All five carriers must be transferred during each 2 minute interval. Flame the hook between each carrier transfer.

10.9.5 The remaining carriers are moved into their corresponding neutralizer tubes at the appropriate time. The carriers may touch the interior sides of the neutralizer tube during the

transfer, but contact should be minimized.

10.9.6 After each carrier is deposited, recap the neutralizer tube and gently shake to facilitate adequate mixing and efficient neutralization.

10.9.7 Record timed events on the Time Recording Sheet for Carrier Transfer Form (see 16.3).

10.9.8 After a minimum of 30 minutes from when the last carrier was deposited, transfer the carriers using a sterile wire hook to a second subculture tube containing 10 mL of the appropriate medium. Sixty secondary tubes are required per test, one for each carrier. Move the carriers in order but the movements do not have to be timed.

10.9.9 Gently shake the entire rack of secondary tubes after all of the carriers have been transferred.

10.9.10 Incubate the neutralizer and secondary subculture tubes for 21 days at 37±1°C.

10.9.11 Report results as growth (+) or no growth (0) on the AOAC Sporicidal Activity Test Results Sheet (see 16.2). A positive result is one in which the medium appears turbid. A negative result is one in which the medium appears clear. Each tube is shaken prior to recording results to determine the presence or absence of turbidity. The neutralizer and secondary subculture tubes for each carrier represent a "carrier set."

10.9.12 A positive result in either the neutralizer or secondary subculture tube is considered a positive result for a carrier set.

10.9.13 If no growth occurs after 21 days at 37±1°C, heat shock all test and control tubes for 20 minutes at 80±2°C and reincubate for 72 hours at 37±1°C. Record results as indicated in 10.8.11.

10.10 Testing Controls: Three sets of control tubes are used to monitor different

aspects of the efficacy test system. They include Media Controls (check of the sterility of the neutralizer and growth medai), Environmental Controls (check of the sterility of the testing environment), and System Controls (check of aseptic technique during the testing and transfer process).

10.10.1 Media Controls: Three unopened, uninoculated neutralizer medium and three unopened, uninoculated subculture medium growth medium tubes are incubated with the sample for 21 days at 37±1°C.

10.10.2 Environmental Controls: Three tubes of subculture medium are left open in the hood while analyst is testing sporicidal activity of product. When analysis is completed, caps are replaced on tubes and tubes are incubated with the sample for 21 days at 37±1°C.

10.10.3 System Controls: Using sterile forceps or sterile needle hooks, transfer 3 sterile carriers into a tube of test disinfectant. Repeat for each type of carrier being tested.

Transfer of system control carriers to neutralizer medium: At the start of the sample test, transfer 1 sterile carrier to a tube of neutralizer medium. After one half of the total sample test spores carriers have been transferred, transfer a second sterile carrier to a tube of neutralizer medium. After all inoculated sample test spore carriers have been transferred, transfer the third sterile carrier to a tube of neutralizer medium.

Transfer of system control carriers to growth medium: At the start of the sample test transfers (into growth medium), transfer 1 sterile carrier from the neutralizer medium tube to a tube of subculture medium growth medium. After one half of the total sample test spores carriers have been transferred, transfer a second sterile carrier to a tube of subculture medium. After all inoculated sample test spore carriers have been transferred, transfer the third sterile carrier to a tube of subculture medium.

All neutralizer and secondary subculture medium tubes are incubated with the sample tubes for 21 days at 37±1°C.

10.11 Confirmation Steps:

10.11.1 A minimum of three positive carrier sets per test, if available, should be confirmed using gram staining, general growth media and VITEK or API analysis. If there are less than three positive carrier sets, then each carrier set will be confirmed. If both tubes are positive in a carrier set, only one tube is selected for confirmatory testing.

10.11.2 For a test with greater than 20 positive carrier sets, confirm at least 20% by gram stain, and a minimum of 4 positive carrier sets by gram staining, general growth media, and API or VITEK analysis (see SOP QC-16, VITEK: Culture Identification Numbers) to ensure the identity of the organism. If both tubes are positive in a carrier set, only one tube is selected for confirmatory testing.

10.11.3 Gram stain reactions, cell morphology, and colony characteristics on general growth media are given in SOP MB-02, Test Microbes: Initiation, Maintenance and Quality Control.

10.11.4 Gram stains are performed on smears taken from the positive culture tubes. For the additional confirmatory tests, a loopful of broth from each selected culture tube is streaked on TSA and incubated for 24±2 hr at 37±1°C. TSA plate is also used for preparing the inoculum for the API strips or the VITEK cards.

10.11.5 The API test and the VITEK analysis should be performed according to the manufacturer's instructions.

10.11.6 If confirmatory testing determines that the identity of the organism was not the test organism, the positive entry (+) on the results sheet must be annotated to indicate a contaminant was present.

11.0 DATA ANALYSIS/CALCULATIONS:

11.1 To calculate average CFU/mL per carrier, average the counts for all plates (those which fall within 30-300 CFU) and divide the count by its dilution.

Counts are taken from one plate in the event that only one of the two plates fall within the 30-300 CFU range.

11.2 To calculate average CFU/carrier, multiply the CFU/mL per carrier by the volume of media used to suspend carrier for sonication or vortexing. Numbers are rounded and only two significant figures are used in calculating averages.

11.3 To calculate average CFU/carrier for all carriers tested, obtain by averaging the average CFU/carrier.

12.0 DATA MANAGEMENT/RECORDS MANAGEMENT:

12.1 Data will be recorded promptly, legibly, and in indelible ink on the forms indicated in section 16.0. Completed forms are archived in notebooks kept in locked file cabinets in D217. Only authorized personnel have access to the locked files. Archived data is subject to OPP's official retention schedule contained in SOP ADM-03 Records and Archives.

13.0 QUALITY CONTROL:

13.1 The OPP Microbiology Laboratory conforms to 40CFR Part 160, Good Laboratory Practices. Appropriate quality control measures are integrated into each SOP.

13.2 For quality control purposes, the required information is documented on the appropriate form(s) (see 16.0).

14.0 NONCONFORMANCE AND CORRECTIVE ACTION:

14.1 Any deviation from the standard protocol and the reason for the deviation will be recorded on the appropriate record sheet (see 16.0); corrective action will be expeditious.

15.0 REFERENCES:

15.1 AOAC Sporicidal Activity Test (Reference: Official Methods of Analysis. 1990. 17th Ed., Association of Official Analytical Chemists, Arlington, VA, Method 966.04). Test methodology is described in detail in the 1993 FDA SOP: FDA Protocol for Testing Sporicidal Activity

16.0 FORMS AND DATA SHEETS:

16.1 AOAC Sporicidal Activity Test Information Sheet

16.2 AOAC Sporicidal Activity Test Results Form

16.3 AOAC Sporicidal Activity Test Time Recording Sheet for Carrier Transfers

16.4 AOAC Sporicidal Activity Test: Performance Controls Results Sheet

16.5 Test Microbe Confirmation Sheet

AOAC Sporicidal Activity Test: Information Sheet

OPP Microbiology Laboratory

TEST INFORMATION/Confirmed by:_____			
EPA Reg. No.		SOP	
Name		Test Date	
Sample No.		Comments:	
Lot No.			
Expiration Date			

TEST PARAMETERS/Confirmed by:_____			
Diluent	Specified	Diluent Used	Hardness Date/Init.
			/ /
Organic Soil	Specified	As Prepared/Date/Init.	
Neutralizer	Specified		
Temperature (°C)	Specified	Chiller Unit Display	Test Tube Waterbath
		Before: After:	Before: After:
	Temperature of Disinfectant in Water Bath		
Contact Time (minutes)	Specified	As Tested	
Type of Carriers	Control #		Preparation #
	1. Porcelain		
	2. Sut. Loops		

TEST MICROBE INFORMATION/Confirmed by:_____	
Test Microbe	
Org. Control No.	
Avg. CFU/Carrier	

REAGENT/MEDIA INFORMATION/Confirmed by:_____			
Reagent/Media	Prep. No.	Reagent/Media	Prep. No.

AOAC Sporicidal Activity Test: Results Form

OPP Microbiology Laboratory

PRODUCT INFORMATION/Confirmed by:_____			
EPA Reg. No.		Test Date	
Name		Test Organism	
Sample No.		Carrier Type	
Lot No.		Comments:	

CARRIER INFORMATION (to be completed by Analyst)	
Carriers	Analyst

TEST RESULTS									
Date Recorded/Initials									
Primary Subculture / Secondary Subculture (carrier)									
1	2	3	4	5	6	7	8	9	10
/	/	/	/	/	/	/	/	/	/
11	12	13	14	15	16	17	18	19	20
/	/	/	/	/	/	/	/	/	/
21	22	23	24	25	26	27	28	29	30
/	/	/	/	/	/	/	/	/	/
31	32	33	34	35	36	37	38	39	40
/	/	/	/	/	/	/	/	/	/
41	42	43	44	45	46	47	48	49	50
/	/	/	/	/	/	/	/	/	/
51	52	53	54	55	56	57	58	59	60
/	/	/	/	/	/	/	/	/	/
Results Summary			Number of Carrier Sets with Growth						
			Number of Carrier Sets without Growth						
Modifications/Comments: * = Positive after heat shock									

AOAC Sporicidal Activity Test: Time Recording Sheet for Carrier Transfers

OPP Microbiology Laboratory

TEST INFORMATION/Confirmed by:_____	
Test Date	
Type of Test/Carrier Type	
Product Reg. No.	
Product Name	
Sample No(s)/ Lot No(s)	
Contact Time	
Organism(s)	

Initials /date	Disinfectant Tube No.	Carrier No.	Carrier Drop Start Time (into the disinfectant)+		Carrier Drop End Time (into the neutralizer media)**		Carrier Transfer (into FTM)
			Clock	Timer*	Clock	Timer	Start Time[1]
Comments: P= Porcelain carriers, SL= Suture loops, BS= *Bacillus subtilis*, FTM = Fluid thioglycollate medium							

AOAC Sporicidal Activity Test: Performance Controls Results Sheet

OPP Microbiology Laboratory

TEST INFORMATION/Confirmed by:_____			
EPA Reg. No.		SOP	
Name		Test Date	
Sample No.		Comments:	
Lot No.			
Expiration Date			

RESULTS			
Date Read/Initials			
Performance Controls			
Type of Controls	Tube #1	Tube #2	Tube #3
Environmental Controls: FTM Tubes			
Media Controls: Neutralizer Tubes			
Media Controls: FTM Tubes			
System Controls: Porcelain Penicylinders			
System Controls: Suture Loops			
Comments:			

REAGENT/MEDIA INFORMATION/Confirmed by:_____			
Reagent/Media	Prep. No.	Reagent/Media	Prep. No.

Test Microbe Confirmation Sheet

OPP Microbiology Laboratory

TEST INFORMATION/Confirmed by:_____			
EPA Reg. No.		Test Date	
Name		Test Organism	
Sample No.		Comments	
Lot No.			

Source: Tube/Plate ID	Date /Initials	Stain Results*	Media Information			Results		
			Name	Prep. No.	Inc. Time/ Temp.	Date/ Initials	Colony Characteristics	API Test/Vitek ID** (if applicable)

* GPC=gram positive cocci, GNR=gram negative rods, AFR=acid fast rods, GPR=gram positive rods

** API or Vitek numerical profile number

*** Use MRME notation for all organisms except *M. bovis*; use MR notation for *M. bovis* (BCG).